by MPM-2 when immunoprecipitated from mitotic cells, suggesting lack of phosphorylation. In support of our hypothesis, non-phosphorylatable 482STOP co-expressed with Orai1 rearranged into near-PM puncta in response to ER Ca^{2+} depletion in mitotic cells, and also significantly rescued mitotic SOCE. A combination of mass spectrometry and site-directed mutagenesis identified S486 and S668 as mitosis-specific phosphorylated residues, and mutation of both to alanine also resulted in partial but significant rescue of SOCE in mitotic cells. Therefore, our data suggest that phosphorylation of S486 and S668 underlies suppression of SOCE during mitosis, although additional phosphorylation sites are likely involved.

517-Pos

Impaired Mitochondria Fail to Ensure Sustained Soce: Possible Mechanism for Decreased Salivary Secretion Under Diabetes

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Xerostomia is a troublesome complication of diabetes mellitus associated with decreased salivation. Previously we showed the diabetes-induced alterations of ACh-mediated $[Ca^{2+}]_{cyt}$ signaling in submandibular salivary gland which provides a major secretion of fluid and electrolytes. Since salivation is initiated by an $InsP_3$-mediated Ca^{2+} release from the ER and subsequently depends on the elevated $[Ca^{2+}]_{cyt}$ maintained by a store-operated Ca^{2+} entry (SOCE), we hypothesized that both processes could be altered under the diabetes contributing to gland dysfunctions. Diabetes was induced by a single i.p. injection of streptozotocin; $[Ca^{2+}]_i$ was measured using fura-2/AM. We found a decrease of the amplitude and deceleration of ACh-induced $[Ca^{2+}]_i$ signals under the diabetes. The increased contribution of mitochondria to the cytosolic calcium clearance in acinar cells was also found under diabetes revealed as: i) an increase in the amount of Ca^{2+}accumulated in mitochondria under basal conditions (by 46%); ii) significantly smaller effect of mitochondrial Ca^{2+} uptake inhibition on the ACh-induced $[Ca^{2+}]_i$ transients in Ca^{2+}-containing extracellular medium (by 69% vs. 29%). Since both SOCE and ER Ca^{2+} refilling are precisely regulated by mitochondria (Kopach et al., 2009), we studied the effectiveness of these processes under diabetes. SOCE induced by short ACh stimulation was increased in diabetic cells (by 70%). Inhibition of mitochondrial Ca^{2+} accumulation equalized SOCE magnitude in control and diabetic cells indicating an increased role of mitochondria to provide positive feedback on SOCE under diabetes. In contrast, during the sustained cells stimulation SOCE was decreased and decelerated under diabetes (~ by 40%) suggesting inability of acinar cells to maintain SOCE under potent agonist stimulation. Concluding, diabetes induces the impairment of intracellular mechanisms responsible for the activation and maintenance of SOCE suppressing mitochondrial Ca^{2+} handling.

518-Pos

Regulation of Vascular Reactivity by Urocortin and Urotensin-II: Role of Store Operated Pathway

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Circulating neuro-hormones, such as Urocortin and Urotensin-II have been demonstrated to critically regulate vascular tone in several arteries. Urocortin was described to induce a strong coronary vasodilatation; beside Urotensin-II was characterized as the most potent mammalian vasoconstrictor identified so far. However, the mechanism of their action is still under debate. The Ca^{2+} independent phospholipase A_2 ($iPLA_2$) dependent activation of store operated Ca^{2+} (SOC) entry have been shown to regulate vascular tone in different arteries. We used vessel myograph, Ca^{2+} imaging, immunocytochemistry and molecular approaches to study the implication of SOC pathway in Urocortin and Urotensin II modulation of rat coronary artery tone.
We observed that Urotensin-II and Urocortin had differential effect on coronary artery. Urocortin induced a potent dose-dependent vasodilatation of agonist-induced coronary contraction. Urocortin activated PKA that inhibited $iPLA_2\beta$ activity and SOC influx in rat SMC. However, Urotensin-II induced a potent vasoconstriction that was sensitive to SOC channel and $iPLA_2$ inhibitors. Urotensin-II produced $iPLA_2\beta$ activation and Ca^{2+} and Mn^{2+} entry in SMC that were inhibited by SOC channels and $iPLA_2\beta$ inhibitors. Interestingly the transfection of SMC with siRNA to Orai1, the pore forming subunit of SOC channels, impaired significantly Urotensin-II induced SOC entry.
These results show that emerging and established transmitter system which can be up- or downregulated in diseases states, regulate differentially the vascular reactivity through the modulation of $iPLA_2\beta$-dependent activation of SOC pathway in coronary artery. This finding is interesting as it gave further information to understand the implication of SOC pathway in physiological and pathological behavior of the coronary artery.

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519-Pos

Calcium Signaling and Prostate Cancer

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Major clinical problem with prostate cancer is the cell's ability to survive and proliferate upon androgen withdrawal. Indeed, deregulated cell proliferation together with the suppression of apoptosis provides the condition for abnormal tissue growth.
Alterations in Ca^{2+} homeostasis have been described to increase proliferation, to induce differentiation or apoptosis. During the last years it has emerged that several members of the TRP family could play an important role in prostate carcinogenesis and even more, some of them have been suggested as a prognostic markers for PCa especially useful in the differential diagnosis.
We were particularly interested by TRPM8 channels since TRPM8 is a target gene of the androgen receptor and its expression strongly increases in prostate cancer. Recent evidence we have obtained indicate that TRPM8 may be expressed not just in the plasma membrane, but also in the endoplasmic reticulum (ER) membrane where TRPM8 may operate as an ER Ca^{2+} release channel. The ''preferred'' TRPM8 localization depends on epithelial cell phenotype (differentiated apical cells vs. non-differentiated basal cells) and on androgen status (androgen-dependent vs. hormone refractory. New results on the differential physiological role of TRPM8 isoforms in prostate cancer cells will be presented.

520-Pos

Frequent Calcium Oscillations Lead to NFAT Activation in Human Immature Dendritic Cells

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Spontaneous Ca2+ oscillations have been reported in certain types of excitable and non-excitable cells. However, the precise molecular mechanism underlying these events and their biological role(s) remain unclear. In the present study we demonstrate for the first time that spontaneous Ca2+ oscillations occur in immature human monocyte-derived dendritic cells and that upon receiving maturation signals via TLRs, the cells lose the high frequency Ca2+ oscillations. We investigated the mechanism and role of the Ca2+ oscillations in immature dendritic cells and found that the inositol-1,4,5-trisphosphate receptor is essential, since oscillations were blocked by pre-treatment of cells with the inositol-1,4,5-trisphosphate receptor antagonist Xestospongin C and 2-APB. A component of the Ca2+ signal is also due to influx from the extracellular environment and may be involved in refilling the intracellular Ca2+ stores. As to their biological role, our results indicate that they are intimately linked to the ''immature'' phenotype and are associated with the translocation of the transcription factor NFAT into the nucleus. In fact, blocking the Ca2+ oscillations with 2-APB or treating the cells with LPS, leading then to undergo maturation, caused NFAT to remain in the cytoplasm. The results presented in this report provide novel insights into the physiology of immature dendritic cells and into the signaling process(es) controlling their maturation.

521-Pos

A Reduction of Spontaneous Beating Rate of Adult Rabbit Pacemaker Cells in Culture is Reversed by RGS2 Overexpression, Gi Inhibition or β-Ar Stimulation

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Genetic manipulation of signaling proteins is an important tool to study signaling mechanisms. While rabbit sinoatrial node cells (SANC) are an excellent model for the study of autonomic signaling, genetic manipulation of freshly isolated rabbit SANC (f-SANC) is not possible. Here we report important characteristics of a cultured rabbit SANC model (c-SANC) that is suitable for manipulation of gene expression. C-SANC generate regular and rhythmic APs at 34 ± 0.5^{o}C, and beat spontaneously at a lower rate (1.35 ± 0.02Hz, n=803) than f-SANC (2.79 ± 0.04Hz, n=203 $p<0.001$). The durations of AP and Ca^{2+}-transient are prolonged in c-SANC. Spontaneous Local Ca^{2+}-Releases (LCR) beneath cell membrane during diastolic depolarization have prolonged period that is highly correlated with c-SANC's reduced spontaneous beating rate.